Reaccumulation of $[K^+]_o$ in the Toad Retina During Maintained Illumination

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ABSTRACT Using K^+-selective microelectrodes, $[K^+]_o$ was measured in the subretinal space of the isolated retina of the toad, *Bufo marinus*. During maintained illumination, $[K^+]_o$ fell to a minimum and then recovered to a steady level that was ~0.1 mM below its dark level. Spatial buffering of $[K^+]_o$ by Müller (glial) cells could contribute to this reaccumulation of K^+. However, superfusion with substances that might be expected to block glial transport of K^+ had no significant effect upon the reaccumulation of K^+. These substances included blockers of g_K (TEA^+, Cs^+, Rb^+, 4-AP) and a gliotoxin (αAAA). Progressive slowing of the rods' Na^+/K^+ pump (perhaps caused by a light-evoked decrease in $[Na^+]_i$) also could contribute to this reaccumulation of K^+ by reducing the uptake of K^+ from the subretinal space. As evidence for a major contribution by this mechanism, treatments designed to prevent such slowing of the pump reversibly blocked reaccumulation. These treatments included superfusion with 2 μM ouabain, or lowering $[K^+]_o$, PO_2, or temperature. It is likely that such treatments inhibit the pump, increase $[Na^+]_i$, and attenuate any light-evoked decrease in $[Na^+]_i$. The results are consistent with the following hypothesis. At light onset, the decrease in rod g_{Na} will reduce the Na^+ influx and the resulting rod hyperpolarization will reduce the K^+ efflux. In combination with these reduced passive fluxes, the continuing active fluxes will lower both $[K^+]_o$ and $[Na^+]_i$, which in turn will inhibit the pump. In support of this hypothesis, the solutions to a pair of coupled differential equations that model changes in both $[K^+]_o$ and $[Na^+]_i$ match quantitatively the time course of the observed changes in $[K^+]_o$ during and after maintained illumination for all stimuli examined.

INTRODUCTION

Illumination of the vertebrate retina evokes a significant decrease in the extracellular potassium ion concentration, $[K^+]_o$, in the subretinal space. This light-evoked decrease in $[K^+]_o$ has been observed in all species examined, including frog (Tomita, 1976; Oakley and Green, 1976), toad (Oakley et al., 1979), mudpuppy (Karwoski and Proenza, 1978), skate (Kline et al., 1978), gecko (Griff

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and Steinberg, 1984), rabbit (Dick and Miller, 1978), and cat (Steinberg et al., 1980). In rod-dominated retinas, the light-evoked decrease in $[K^+]_o$ seems to be produced primarily by the rod photoreceptors themselves (Matsuura et al., 1978; Oakley et al., 1979; Steinberg et al., 1980). With maintained illumination, $[K^+]_o$ falls to a minimum and then begins to recover back toward the dark-adapted level (this latter process is termed the "reaccumulation" of K^+). With continuous illumination, $[K^+]_o$ eventually recovers by an amount equal to 65–85% of its initial decrease at light onset (Steinberg et al., 1980; Oakley and Steinberg, 1982; Oakley, 1983). Following termination of maintained illumination, there is a transient increase in $[K^+]_o$ above the dark-adapted baseline (an overshoot) that nearly is a mirror image of the transient decrease in $[K^+]_o$ at light onset.

The reaccumulation of K^+ during maintained illumination is likely to be of physiological significance. For example, membrane voltage in retinal pigment epithelial (RPE) cells is determined in part by $[K^+]_o$ in the subretinal space (Miller and Steinberg, 1977), and in frog, changes in RPE membrane voltage follow $[K^+]_o$ both during and after maintained illumination (Oakley and Steinberg, 1982). Any RPE transport processes that depend either on membrane voltage or on $[K^+]_o$ (e.g., Miller and Steinberg, 1979) will be affected by the reaccumulation of K^+. In addition, although retinal sensitivity is affected by changes in $[K^+]_o$ (Dowling and Ripps, 1976), the reaccumulation of K^+ makes it unlikely that maintained changes in sensitivity will be caused by $[K^+]_o$ (Steinberg et al., 1980). One of the most significant aspects of the reaccumulation of K^+ is that it helps to re-establish a stable extracellular environment, that is, a $[K^+]_o$ homeostasis.

Two quite different mechanisms could contribute to the reaccumulation of K^+ during maintained illumination. One of these mechanisms is the spatial buffering of $[K^+]_o$ by glial cells, whereby the high K^+ conductance, g_K, of glial cells will allow K^+ to enter glial cells in regions of high $[K^+]_o$ and exit in regions of low $[K^+]_o$. In some tissues, such as drone retina (Coles and Tsacopoulos, 1979; Gardner-Medwin et al., 1981; Coles and Orkand, 1983), cat cortex (Dietzel et al., 1980), rat neocortex (Gardner-Medwin, 1983; Gardner-Medwin and Nicholson, 1983), and rat optic nerve (Yamate et al., 1983), spatial buffering of $[K^+]_o$ by glial cells is involved in the regulation of $[K^+]_o$. In fact, during maintained depolarizing stimulation of several of these tissues, spatial buffering of $[K^+]_o$ by glial cells can produce changes in $[K^+]_o$ similar to those observed in the vertebrate retina during and after maintained illumination (but of opposite polarity).

Another mechanism that could contribute to the reaccumulation of K^+ in the vertebrate retina involves changes in the activity of the Na^+/K^+ pump in the rod membrane, or in other cells bordering on the subretinal space (Steinberg et al., 1980; Oakley, 1983, 1984). In tissues as diverse as cat cortex (Heinemann and Lux, 1975, 1977; Nicholson et al., 1978), frog ventricular muscle (Kunze, 1977; Martin and Morad, 1982; Kline and Kupersmith, 1982), guinea pig hippocampus (Benninger et al., 1980), and rat sympathetic ganglia (Galvan et al., 1979), changes in the activity of Na^+/K^+ pumps are involved in the regulation of $[K^+]_o$. During maintained depolarizing stimulation, an increase in the activity of the Na^+/K^+ pump, caused by an increase in $[Na^+]_i$ (Cohen et al., 1982; Ballanyi et

al., 1983), produces changes in $[K^+]_o$ similar to those observed in the retina during and after maintained illumination (but of opposite polarity). By analogy with these tissues, inhibition of the rods' Na^+/K^+ pump during maintained illumination, perhaps caused by a decrease in $[Na^+]_i$, could contribute to the reaccumulation of K^+ (Oakley, 1983).

In previous experiments (Oakley, 1983), it was found that superfusion of the isolated retina of the toad with 2.0 mM Ba^{2+} reversibly blocked the reaccumulation of K^+. This result did not help to determine the relative contributions of the two mechanisms that were suggested (above) to be involved in the reaccumulation process, since Ba^{2+} could block g_K in glial cells and thus block the movement of K^+ through glial cells, but Ba^{2+} also could have direct inhibitory effects on the Na^+/K^+ pump at the relatively high concentrations used (Ellory et al., 1983; Oakley, 1983).

The experiments reported in this paper were designed to affect the two different mechanisms selectively, in order to provide information regarding the relative contributions of these mechanisms to the reaccumulation of K^+ during maintained illumination. Experiments designed to affect the spatial buffering of $[K^+]_o$ by glial cells had little effect on the reaccumulation process, while experiments designed to affect Na^+/K^+ pumps blocked reversibly the reaccumulation process. On the basis of these data, a model of the ionic mechanisms involved in the regulation of $[K^+]_o$ was developed, and this model can be used to explain quantitatively the kinetics of the observed changes in retinal $[K^+]_o$ during and after maintained illumination under a wide variety of experimental conditions.

METHODS

Preparation

All experiments were performed on the isolated retina preparation of the toad, *Bufo marinus*, as described in detail recently (Oakley, 1983). Briefly, the isolated retina was pinned, receptor side up, in a small chamber (~0.25 ml vol) that had a transparent bottom. This chamber was placed on the stage of a compound microscope, and the retina was viewed using infrared illumination (>850 nm) and an image converter. The experiments were performed at room temperature (22–24°C), unless otherwise noted.

Solutions and Experimental Conditions

During an experiment, the retina was superfused (1.5–2.0 ml/min) with an oxygenated Ringer's solution that had the following composition (in mM): 110 NaCl, 2.4 KCl, 0.9 $CaCl_2$, 1.3 $MgCl_2$, 0.22 NaH_2PO_4, 2.78 Na_2HPO_4, 5.6 glucose, 0.01 EDTA, and 0.003 phenol red. The pH of this solution was 7.8. Various test solutions were prepared by slight alterations in the control solution. The effects of Rb^+ were investigated by equimolar substitution of RbCl for 2.4 mM KCl. The effects of Cs^+ or tetraethylammonium ion (TEA^+) were investigated by equimolar substitution of 1.0–10.0 mM CsCl or 5.0–10.0 mM TEA-Cl for NaCl. The effects of 4-aminopyridine (4-AP) were investigated by the addition of 1.0–2.0 mM 4-AP to the control solution. Solutions having lowered K^+ were made by equimolar substitution of NaCl for KCl. A solution containing DL-α-aminoadipic acid (αAAA) was made by equimolar substitution of αAAA for 5.0–10.0 mM NaCl. Ouabain (1.0–10.0 μM) was simply added to the control solution.

In several experiments, the retinal temperature was lowered by bathing the retina in cooled Ringer's solution. The temperature of the solution surrounding the retina was measured with a miniature thermistor probe (model 514; Yellow Springs Instrument Co., Yellow Springs, OH). In other experiments, retinal PO_2 was lowered by bubbling the Ringer's solution with N_2 instead of with O_2. Although this solution nominally had a PO_2 close to zero, it was likely that a small amount of O_2 could diffuse into the solution when it was exposed to the room atmosphere in the chamber. The exact PO_2 of the solution surrounding the retina was not measured under these conditions.

Electrodes and Recording

Double-barreled, K^+-selective microelectrodes were used to measure $[K^+]_o$. One barrel was an ion-selective electrode and contained a K^+-selective liquid (477317, Corning Medical Products, Medfield, MA; or 60031, Fluka Chemical Corp., Hauppauge, NY) in its tip; the remainder of this barrel was filled with 0.1 M KCl. The other barrel was a reference electrode filled with 1.0 M LiCl (Steinberg et al., 1980). The double-barreled microelectrodes were beveled on a surface embedded with diamond dust (Brown and Flaming, 1979). After beveling, the resistance of the reference barrel was 25–40 MΩ.

Each K^+-selective microelectrode was advanced toward the receptor surface from above under visual control, until the electrode tip just made contact with a rod outer segment. The electrode then was advanced into the preparation in 2-μm steps, using a piezoelectric positioning system (Burleigh Instruments, Inc., Fishers, NY). In most experiments, the electrode tip was positioned ~40–60 μm below the receptor surface, at a depth where both the amplitude and the initial rate of change of the light-evoked decrease in $[K^+]_o$ were maximal (Oakley et al., 1979).

Immediately after an experiment, each K^+-selective microelectrode was calibrated in solutions having varying $[K^+]$ and a fixed background of 110 mM $[Na^+]$. The calibration data were fitted by an equation of the form:

$$V_{K^+} = A \log_{10}\left([K^+]_o + \frac{[Na^+]_o}{S}\right) + V_o, \tag{1}$$

where V_{K^+} is the differential potential between the two barrels (K^+-selective barrel positive), A is the logarithmic slope, S is the selectivity coefficient for K^+ over Na^+, and V_o is a constant (Walker, 1971; Oakley, 1983). The value of A was 55–58 mV/decade, while the value of S was 60–70 for Corning electrodes and was 1,000–2,000 for Fluka electrodes. Once the electrode calibration curve was determined, it was possible to convert entire digitized waveforms of V_{K^+} into waveforms of $[K^+]_o$ by rearranging Eq. 1 into the form:

$$[K^+]_o = 10^{\left(\frac{V_{K^+} - V_o}{A}\right)} - \frac{[Na^+]_o}{S}. \tag{2}$$

The Corning K^+ ion exchanger has a greater sensitivity for Rb^+ than for K^+ (Wise et al., 1970), so the Corning electrodes could be used as Rb^+-selective electrodes to measure $[Rb^+]_o$ in the retina. However, the Corning ion exchanger also has a much greater sensitivity for TEA^+, Cs^+, and 4-AP than for K^+ (Wise et al., 1970; Neher and Lux, 1973; B. Oakley, unpublished observations), so it was not possible to use the Corning electrodes to measure $[K^+]_o$ in the presence of these interfering ions. Instead, the Fluka K^+ cocktail, which is based on valinomycin (Oehme and Simon, 1976; Wuhrmann et al., 1979), was used. At the extracellular concentration of K^+ (2.4 mM), the Fluka electrodes had little response to the concentrations of TEA^+ and 4-AP used in these experiments. The Fluka electrodes had a larger response to Cs^+, however, since they are nearly as sensitive to Cs^+ as to K^+ (Oehme and Simon, 1976). Under control conditions, no differences were

observed between the light-evoked decreases in $[K^+]_o$ measured by either type of electrode. However, the light-evoked changes in V_{K^+} usually were larger in amplitude when the Fluka electrodes were used, since there was less interference from extracellular Na^+; the resistance of the Fluka electrodes also was larger.

All microelectrode voltages were measured using capacity-compensated preamplifiers having input resistances of 10^{15} Ω. These voltages were referenced to a Ag/AgCl electrode that was connected electrically via a KCl-agar bridge to the solution bathing the retina. The electrode voltages were amplified, displayed on both an oscilloscope and a chart recorder, and recorded on an instrumentation tape recorder for off-line analysis. The recordings were digitized using a laboratory computer system and plotted on a digital plotter (Oakley, 1983).

Light Stimulation

The retina was stimulated with 500 nm light, delivered through the microscope condenser. The stimulus diameter in the plane of the retina was ~2 mm, and all microelectrode recordings were made from the center of the stimulated region. The stimulus duration could be varied using an electromagnetic shutter. The stimulus irradiance was attenuated with calibrated neutral density filters. For all responses illustrated, the stimulus irradiance was 1.3 log quanta s^{-1} μm^{-2}.

RESULTS

Experiments Designed to Affect Glial Cells

Spatial buffering of $[K^+]_o$ by glial cells could contribute to the reaccumulation of K^+ during maintained illumination. Glial cells are known to have a large g_K, and thus glial cells may be able to regulate $[K^+]_o$ by providing a current-mediated transcellular flux of K^+ (e.g., Orkand et al., 1966; Varon and Somjen, 1979; Gardner-Medwin, 1983). The differential hyperpolarization of Müller (glial) cells by the light-evoked decrease in $[K^+]_o$ could lead to current flow and the transcellular movement of K^+ through Müller cells. Such movement of K^+ might possibly lead to a reaccumulation of K^+ similar to that actually observed. If this is the case, then experimental conditions designed to interfere with Müller cells should block the reaccumulation process.

Specifically, blocking g_K in Müller cells should abolish any fluxes of K^+ through these cells. In a variety of cell types, substances such as Cs^+, Rb^+, TEA^+, and 4-AP are known to block K^+ conductances (Hille, 1967; Narahashi, 1974; Hagiwara and Takahashi, 1974; Hagiwara et al., 1976; Meves and Pichon, 1977; Edwards, 1982). It is likely that these substances also block the g_K of Müller cells, since they abolish the slow PIII component of the electroretinogram (Winkler and Gum, 1981; B. Oakley, manuscript submitted for publication), which is thought to be generated by the Müller cells' hyperpolarizing response to the light-evoked decrease in $[K^+]_o$ (Witkovsky et al., 1975; Fujimoto and Tomita, 1979). These substances have little effect on the decrease in $[K^+]_o$ evoked by brief flashes of light (B. Oakley, manuscript submitted for publication).

Moreover, the gliotoxin αAAA is known to disrupt Müller cell membranes (Szamier et al., 1981; Bonaventure et al., 1981; Welinder et al., 1982; Zimmerman and Corfman, 1984), and αAAA appears to disrupt the ability of Müller

cells to respond to changes in $[K^+]_o$ (Karwoski et al., 1982). If this is the case, then application of αAAA also might be expected to disrupt the ability of Müller cells to participate in spatial buffering of $[K^+]_o$ during maintained illumination.

In the first set of experiments designed to affect Müller cells, the retina was bathed in various substances known to block g_K in a wide variety of cells. The results of these experiments are summarized in Fig. 1. Each part of Fig. 1 illustrates the effect of a different test substance. With each test substance, a control response to a 180-s period of illumination is shown, as well as a response to the same stimulus recorded while bathing the retina in the test substance. In Fig. 1*A*, 4-AP was used. Even after bathing the retina in 1.0 mM 4-AP for 26 min, there was no detectable effect upon the changes in $[K^+]_o$. Similar results were obtained with 2.0 mM 4-AP. In Fig. 1*B*, TEA^+ was used. In 10.0 mM TEA^+, the light-evoked changes in $[K^+]_o$ were slightly different than under control conditions, in that they were reduced to ~75% of their control amplitudes. However, there still was a significant reaccumulation and overshoot in 10.0 mM TEA^+. In Fig. 1*C*, Cs^+ was used. The change in V_{K^+} was much smaller during superfusion with 1.0 mM Cs^+; however, this voltage represented almost the same change in $[K^+]_o$, because of the significant response of the K^+-selective microelectrode (Fluka K^+ cocktail) to Cs^+. Similar results were obtained with concentrations of Cs^+ up to 5.0 mM. In Fig. 1*D*, K^+ was replaced completely by Rb^+. As observed elsewhere (B. Oakley, manuscript submitted for publication), there was a light-evoked change in $[Rb^+]_o$ of essentially the same amplitude as the change in $[K^+]_o$. Moreover, there was a reaccumulation of Rb^+ during maintained illumination, and an overshoot of $[Rb^+]_o$ following light offset.

Overall, the results illustrated in Fig. 1 can be summarized by stating that substances known to block g_K in a wide variety of cells had no significant effect upon the reaccumulation of K^+ during maintained illumination. The smaller amplitude of the light-evoked decrease in $[K^+]_o$ observed during superfusion with TEA^+ or Cs^+ may have been due to effects of these substances upon Müller cells or upon the rods themselves (e.g., Fain and Quandt, 1980). However, none of these substances significantly affected the conductance in rods responsible for the light-evoked decrease in $[K^+]_o$, which is consistent with other findings (B. Oakley, manuscript submitted for publication).

In another series of experiments designed to affect glial cells, the retina was bathed in various concentrations of the gliotoxin αAAA. The results illustrated in Fig. 2 were obtained during an experiment in which 10.0 mM αAAA was used. In the upper trace of Fig. 2, a control response is shown. In the lower trace of Fig. 2, a response is shown that was recorded after 3 min of superfusion with 10.0 mM αAAA. In 10.0 mM αAAA, the changes in $[K^+]_o$ were reduced to ~75% of their control amplitudes. This result could have been produced by a direct effect of αAAA upon the Müller cells or by neurotoxic effects of the DL-isomer upon the rods (see Zimmerman and Corfman, 1984). Nevertheless, there was a reaccumulation of K^+ during maintained illumination, as well as an overshoot at light offset. Qualitatively similar responses were observed even after 60 min of continuous superfusion with 10.0 mM αAAA (data not illustrated).

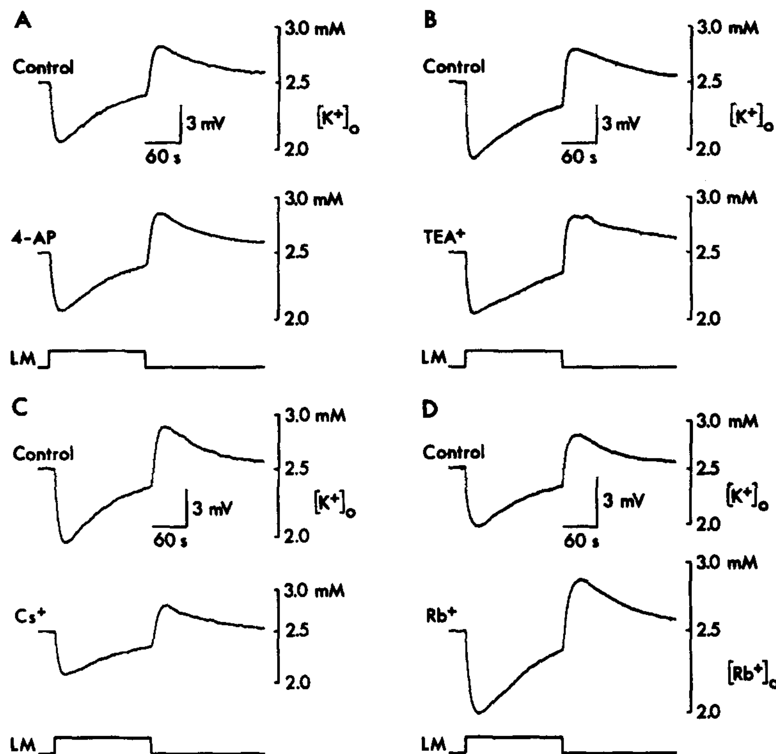

FIGURE 1. Effects of blockers of g_K upon the light-evoked changes in $[K^+]_o$. Each panel of this figure shows both a control response and a response recorded during superfusion with a blocker of g_K. All responses were evoked by light stimuli having durations of 180 s, as indicated by the traces labeled LM (light monitor). (*A*) Effects of 4-AP. The lower response was recorded after 26 min of superfusion with 1.0 mM 4-AP. (*B*) Effects of TEA^+. The lower response was recorded after 4 min of superfusion with 10.0 mM TEA^+. (*C*) Effects of Cs^+. The lower response was recorded after 10 min of superfusion with 1.0 mM Cs^+. The light-evoked decrease in V_{K^+} was smaller during superfusion with Cs^+, because of the selectivity of the K^+-selective microelectrode for K^+ over Cs^+ of only 1.2:1. However, this change in V_{K^+} represented a change in $[K^+]_o$ of nearly the same amplitude as under control conditions, after taking into account the electrode selectivity. (*D*) Effects of Rb^+. The lower response was recorded after 20 min of superfusion with a solution in which K^+ was replaced by Rb^+. Since the K^+-selective microelectrode was more sensitive to Rb^+ than to K^+, this waveform represents a light-evoked change in $[Rb^+]_o$.

Experiments Designed to Affect the Rods' Na^+/K^+ Pump

A decrease in the rate of the Na^+/K^+ pump in the rods could contribute to the reaccumulation of K^+ during maintained illumination. By analogy with other cell types, it was suggested that a light-evoked decrease in $[Na^+]_i$ could progressively inhibit the Na^+/K^+ pump and thus decrease the active uptake of K^+ during this

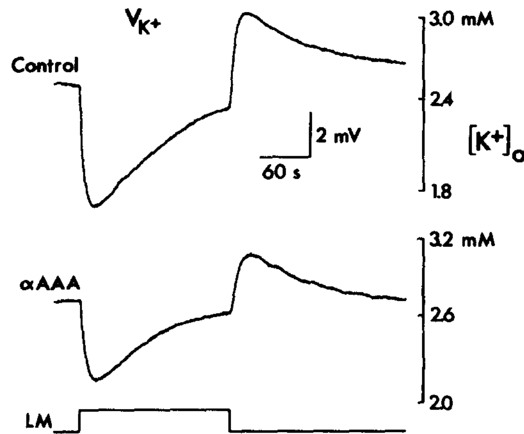

FIGURE 2. Effects of αAAA. These responses were evoked by 180-s light stimuli (as indicated by the trace labeled LM). The upper response was recorded under control conditions, and the lower response was recorded 3 min after starting continuous superfusion with a solution containing 10.0 mM αAAA. The level of $[K^+]_o$ in the dark increased by 0.2 mM during the first few minutes of superfusion with αAAA solution. The effects of αAAA were largely irreversible.

time period, leading to the observed reaccumulation (Oakley, 1983). If this is the case, then the experimental conditions designed to inhibit the pump should have several effects. If the pump is inhibited, then the reduced active efflux of Na^+ should not produce as large a decrease in $[Na^+]_i$ during time periods when the passive influx of Na^+ is reduced. Moreover, if the pump is inhibited, the level of $[Na^+]_i$ is likely to increase (Chapman et al., 1983) in the dark. In other cell types, $[Na^+]_i$ stimulates the pump in a sigmoidal manner (Garay and Garrahan, 1973; Nelson and Blaustein, 1980; Saito and Wright, 1982). Thus, it is possible that any light-evoked decrease in $[Na^+]_i$ will take place over a region of higher $[Na^+]_i$, where the pump is relatively less sensitive to changes in $[Na^+]_i$. Taking into account all of these effects, it seems likely that any progressive inhibition of the pump occurring during maintained illumination should be minimized if the pump already is inhibited.

In the first set of experiments designed to affect the Na^+/K^+ pump in the rods, the retinal temperature was lowered. In general, temperature is known to have significant effects on the rate of active transport by Na^+/K^+ pumps (e.g., Skou, 1965; Glynn and Karlish, 1975). In Fig. 3, effects of lowering retinal temperature are illustrated. In Fig. 3*A*, three responses are shown: a control response recorded at 22°C, a response recorded after 23 min at 9°C, and a response recorded after re-warming the retina to 22°C. Cooling the retina abolished reversibly the reaccumulation of K^+ during maintained illumination and also abolished the overshoot of $[K^+]_o$ following light offset. In Fig. 3*B*, the first 70 s of the control (22°C) and low temperature (9°C) responses have been scaled for equal amplitudes and superimposed. The initial slope of the normalized response at 9°C was slightly smaller in magnitude than the control response. In Fig. 3*C*, the gradient

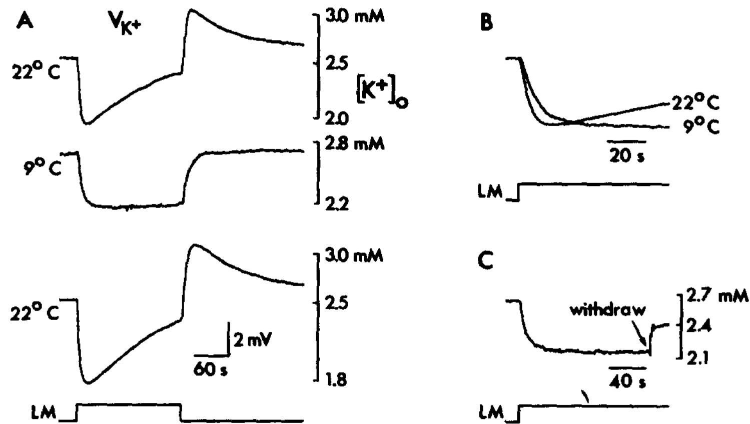

FIGURE 3. Effects of lowering retinal temperature. (*A*) These three responses were evoked by identical stimuli having durations of 180 s (as indicated by the trace labeled LM). The upper response was recorded under control conditions at 22°C, the middle response was recorded 23 min after cooling the retina to 9°C, and the lower response was recorded 28 min after warming the retina back to 22°C. The level of $[K^+]_o$ in the dark increased by 0.2 mM during the first few minutes of cooling. The 2-mV calibration bar applies to the responses in parts *A* and *C*. (*B*) The initial 70 s of the first control response (22°C) and the low temperature response (9°C) have been scaled for equal peak amplitudes and superimposed. (*C*) In another retina that had been maintained at 10°C for 10 min, the tip of the K^+-selective microelectrode was withdrawn into the bathing solution ~150 s after the onset of a maintained stimulus.

of $[K^+]_o$ during maintained illumination was measured in another retina at 10°C. Approximately 150 s after the onset of maintained illumination, the tip of the K^+-selective microelectrode was withdrawn into the bathing solution (having a $[K^+]$ of 2.4 mM). The change in electrode voltage showed that the steady level of $[K^+]_o$ in the subretinal space was below that of the bathing solution during maintained illumination. The presence of this gradient indicated that an active uptake mechanism was still functioning (e.g., Martin and Morad, 1982); that is, the Na^+/K^+ pump was not completely inhibited.

In another series of experiments, the Na^+/K^+ pump in the rods was inhibited by bathing the retina in ouabain (e.g., Skou, 1965; Frank and Goldsmith, 1967; Glynn and Karlish, 1975). Various concentrations of ouabain were tried, in order to find one that did not irreversibly abolish the light-evoked decrease in $[K^+]_o$ (Oakley et al., 1979). As shown in Fig. 4, a concentration of 2 μM was found to produce reversible effects on the changes in $[K^+]_o$ during and after maintained illumination, similar to the effects of lowered temperature. It was not totally unexpected to find that ouabain was effective at such a low concentration, since in previous experiments, Torre (1982) found that the Na^+/K^+ pump in toad rods was affected significantly by 3 μM strophanthidin, a cardioactive steroid with effects similar to ouabain. In Fig. 4*A*, three responses are shown: a control response, a response recorded after 17 min of bathing the retina in 2 μM ouabain

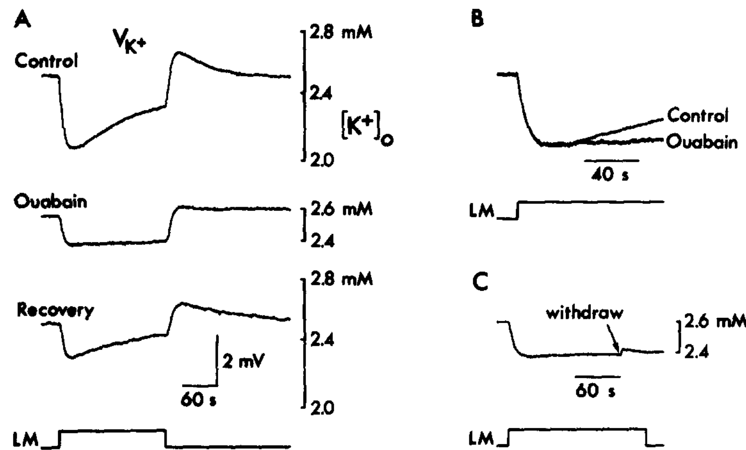

FIGURE 4. Effects of ouabain. (*A*) These three responses were evoked by identical stimuli having durations of 180 s (as indicated by the trace labeled LM). The upper response was recorded under control conditions, the middle response was recorded 17 min after starting to superfuse the retina with a solution containing 2 μM ouabain, and the lower response was recorded 95 min after returning to superfusion with control solution. The level of $[K^+]_o$ in the dark increased by 0.1 mM during the first few minutes of superfusion with ouabain solution. The 2-mV calibration bar applies to the responses in parts *A* and *C*. (*B*) The initial 104 s of the first control response and the response in ouabain solution have been scaled for equal peak amplitudes and superimposed. (*C*) In another retina that had been superfused with 2 μM ouabain for 19 min, the tip of the K^+-selective microelectrode was withdrawn into the bathing solution ~150 s after the onset of a maintained stimulus.

solution, and a response recorded 95 min after switching back to control solution. Ouabain reversibly attenuated both the reaccumulation of K^+ during maintained illumination and the overshoot in $[K^+]_o$ at light offset. In Fig. 4*B*, the first 70 s of the control response and the response in ouabain solution have been scaled for equal amplitudes and superimposed. The initial slope of the normalized response in ouabain was essentially the same as it was under control conditions. In Fig. 4*C*, the gradient of $[K^+]_o$ was measured in another retina that had been bathed in 2 μM ouabain for 19 min. As in Fig. 3*C*, the microelectrode was withdrawn into the bathing solution ~150 s after stimulus onset. The change in the electrode voltage indicated that the level of $[K^+]_o$ in the subretinal space was slightly below that of the bathing solution during maintained illumination. The presence of this small gradient indicated that the Na^+/K^+ pump was not completely inhibited, since this active uptake mechanism could still decrease $[K^+]_o$ slightly below the level of the bathing solution.

In yet another attempt to affect selectively the Na^+/K^+ pump in the rods, the retina was bathed in a solution having 0 mM K^+ (K^+-free solution). As with the other treatments (lowering temperature, ouabain), low $[K^+]_o$ should inhibit the Na^+/K^+ pump (e.g., Garay and Garrahan, 1973; Saito and Wright, 1982). The effects of K^+-free solution are shown in Fig. 5. In Fig. 5*A*, three responses are

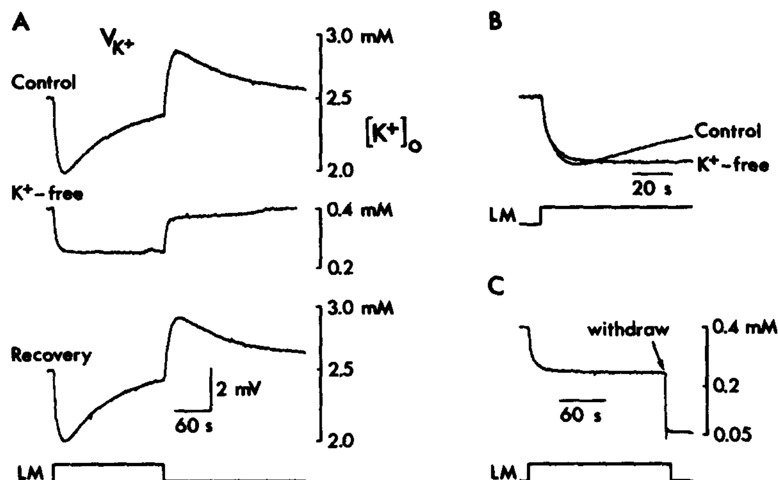

FIGURE 5. Effects of K^+-free solution. (*A*) These three responses were evoked by identical stimuli having durations of 180 s (as indicated by the trace labeled LM). The upper response was recorded under control conditions, the middle response was recorded 20 min after starting to superfuse the retina with K^+-free solution, and the lower response was recorded 13 min after returning to superfusion with control solution. The level of $[K^+]_o$ in the dark decreased to 0.4 mM during the first 20 min of superfusion with K^+-free solution. The 2-mV calibration bar applies to the responses in parts *A* and *C*. (*B*) The initial 70 s of the first control response and the response in K^+-free solution have been scaled for equal peak amplitudes and superimposed. (*C*) In another retina that had been superfused with K^+-free solution for 27 min, the tip of the K^+-selective microelectrode was withdrawn into the bathing solution ~170 s after the onset of a maintained stimulus.

shown: a control response, a response recorded after bathing the retina with K^+-free solution for 20 min, and a response recorded 13 min after restoring the normal $[K^+]_o$. Bathing the retina in K^+-free solution lowered $[K^+]_o$ in the subretinal space to ~0.4 mM in 20 min. Lowering $[K^+]_o$ reversibly abolished the reaccumulation of K^+ during maintained illumination and also abolished the overshoot of $[K^+]_o$ following light offset. In Fig. 5*B*, the first 70 s of the control response and the response recorded in K^+-free solution have been scaled for equal amplitudes and superimposed. The initial slope of the normalized response in K^+-free solution was essentially the same as it was under control conditions. In Fig. 5*C*, the gradient of $[K^+]_o$ during maintained illumination was measured in another retina that had been bathed in K^+-free solution for 27 min. As in Figs. 3*C* and 4*C*, the microelectrode was withdrawn into the bathing solution (nominally 0.0 mM $[K^+]$) ~170 s after the onset of maintained illumination. The change in the electrode voltage indicated that the steady level of $[K^+]_o$ in the subretinal space was higher than that of the bathing solution during maintained illumination. The presence of this large gradient suggests that the retina was continuously losing K^+ to the bathing solution, as would be the case if the Na^+/K^+ pump was very inhibited, so that there was little active uptake of K^+.

In one final series of experiments designed to affect the Na^+/K^+ pump in rods, retinal PO_2 was lowered. Since the pump has a high demand for O_2, the pump should become inhibited under conditions of anoxia (Kimble et al., 1980). In these experiments, several values of PO_2 were used. A solution bubbled with room air had a negligible effect on the changes in $[K^+]_o$ during and after maintained illumination. However, a solution bubbled with N_2 (termed "low PO_2 solution") had significant effects on these changes in $[K^+]_o$, as shown in Fig. 6. In Fig. 6*A*, three responses are shown: a control response, a response recorded after 22 min of bathing the retina in low PO_2 solution, and a response recorded 24 min after switching back to control solution. Low PO_2 reversibly attenuated both the reaccumulation of K^+ during maintained illumination and the overshoot in $[K^+]_o$ at light offset. In Fig. 6*B*, the first 70 s of the control response and the response in low PO_2 solution have been scaled for equal amplitudes and superimposed. The initial slope of the normalized response in low PO_2 solution was slightly less than it was under control conditions. In Fig. 6 *C*, the gradient of $[K^+]_o$ was measured in another retina that had been bathed in low PO_2 solution for 14 min. The microelectrode was withdrawn into the bathing solution ~150 s after stimulus onset. The change in the electrode voltage indicated that the level of $[K^+]_o$ in the subretinal space was essentially the same as that of the

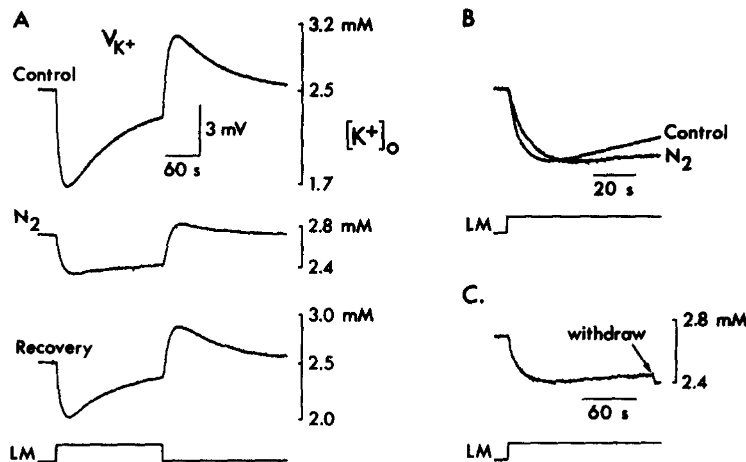

FIGURE 6. Effects of lowering PO_2. (*A*) These three responses were evoked by identical stimuli having durations of 180 s (as indicated by the trace labeled LM). The upper response was recorded under control conditions, the middle response was recorded 22 min after starting to superfuse the retina with a low PO_2 solution, and the lower response was recorded 24 min after restoring the normal PO_2. The level of $[K^+]_o$ in the dark increased by 0.2 mM during the first few minutes of superfusion with low PO_2 solution. The 3-mV calibration bar applies to the responses in parts *A* and *C*. (*B*) The initial 70 s of the first control response and the response in low PO_2 solution have been scaled for equal peak amplitudes and superimposed. (*C*) In another retina that had been bathed in low PO_2 solution for 14 min, the tip of the K^+-selective microelectrode was withdrawn into the bathing solution ~150 s after the onset of a maintained stimulus.

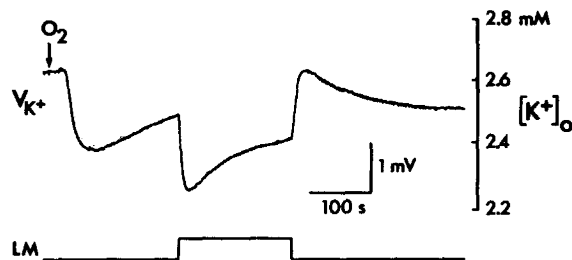

FIGURE 7. Changes in V_{K^+} evoked by an increase in PO_2 and light. The retina had been bathed in low PO_2 solution for 19 min prior to the start of this record. At the time indicated by the arrow, the solution bathing the retina was switched to the control (oxygenated) solution. Approximately 3 min later, a 180-s light stimulus was given, as indicated by the trace labeled LM.

bathing solution during maintained illumination. This result suggests that the Na^+/K^+ pump was very inhibited, since this active uptake mechanism could not decrease $[K^+]_o$ below the level of the bathing solution.

In the experiments in which PO_2 was varied, complex changes in $[K^+]_o$ were observed when control PO_2 was restored after a period of time during which the retina had been bathed in low PO_2 solution, as shown in Fig. 7. When the retina suddenly was exposed to O_2, the level of $[K^+]_o$ fell abruptly, and then there was a reaccumulation of K^+ back toward the control level. This reaccumulation of K^+ was similar in time course to the reaccumulation of K^+ during maintained illumination, as shown by the response to a 180-s light stimulus, delivered as the level of $[K^+]_o$ approached the control level. Similar changes in $[K^+]_o$ were observed when the retinal temperature was suddenly increased after a prolonged period of low temperature (data not illustrated), and when normal $[Ca^{2+}]_o$ was restored after a prolonged period of 1/10 normal $[Ca^{2+}]_o$ (Oakley, 1984).

It is possible that the changes in $[K^+]_o$ illustrated in Fig. 7 were produced as follows. Inhibition of the pump would cause an increase in $[Na^+]_i$, so that when the normal PO_2 was restored, the pump would be stimulated by the high $[Na^+]_i$, causing an increased uptake of K^+ and a decrease in $[K^+]_o$. As $[Na^+]_i$ would subsequently fall, the pump would slow and allow $[K^+]_o$ to reaccumulate. Similar effects of altered $[Na^+]_i$ were postulated to explain the effects of changing $[Ca^{2+}]_o$ observed previously (Oakley, 1984). When ouabain solution was used, the level of $[K^+]_o$ did not change as abruptly as in Fig. 7, presumably since the effects of ouabain were slower to reverse. When 0 mM $[K^+]$ solution was used, any similar changes in $[K^+]_o$ were not observed, since the level of $[K^+]_o$ was increasing rapidly as the control conditions were restored.

DISCUSSION

The experiments reported in this paper investigated the relative contributions of two quite different mechanisms to the reaccumulation of K^+ during maintained illumination. One mechanism involved the spatial buffering of $[K^+]_o$ by glial cells. However, the reaccumulation of K^+ during maintained illumination was not affected significantly by conditions that were designed to block g_K in glial cells.

Moreover, a gliotoxin, which in other experiments seemed to disrupt the ability of glial cells to respond to changes in $[K^+]_o$ (Karwoski et al., 1982), had little effect upon the reaccumulation process. Thus, these data seem to indicate that the contribution of spatial buffering is minimal in this preparation. However, any more definitive assessment of the contribution of spatial buffering must await a direct demonstration that the glial cells actually were affected in the assumed manner by the conditions employed in these experiments.

The other mechanism that was examined in these experiments involved progressive inhibition of the rods' Na^+/K^+ pump during maintained illumination, and the experimental data are consistent with the idea that a major contribution to the reaccumulation of K^+ comes from this mechanism. Experimental conditions designed to affect Na^+/K^+ pumps selectively had significant effects on the changes in $[K^+]_o$ during maintained illumination. It is not likely that Na^+/K^+ pumps on cells other than the rods are participating in the reaccumulation process in the isolated retina preparation. It will be shown (below) that the reaccumulation process most likely involves the response of the Na^+/K^+ pump to some event other than just the decrease in $[K^+]_o$ (such as a decrease in $[Na^+]_i$). Since the reaccumulation of K^+ is not affected by blocking synaptic transmission to second-order neurons with aspartate (Oakley, 1983), it seems unlikely that events are occurring in second-order neurons that would affect their Na^+/K^+ pumps in the necessary manner. In addition, it seems unlikely that the Na^+/K^+ pumps on Müller cells (Stirling and Lee, 1980) would be affected in the necessary manner, since these pumps presumably respond only to the light-evoked decrease in $[K^+]_o$.

A Possible Mechanism for the Reaccumulation Process

Matsuura, Miller, and Tomita (1978) developed and tested a model (termed here the MMT model) of the light-evoked decrease in $[K^+]_o$, and this model was supported in a subsequent study by Oakley et al. (1979). According to this model, the light-evoked hyperpolarization of the rod membrane, caused by a decrease in Na conductance, g_{Na}, reduced the driving force on K^+ across the rod membrane and thus reduced the passive efflux of K^+ out of the rods. The activity of the Na^+/K^+ pump in the rod membrane was assumed to be regulated by $[K^+]_o$, in that the active influx of K^+ into the rod was assumed to vary linearly with $[K^+]_o$. The difference between the active influx and the passive efflux of K^+ was the net uptake of K^+ from the extracellular space, and it was this net uptake that was responsible for decreasing $[K^+]_o$. The MMT model yielded a linear, first-order differential equation describing the kinetics of the light-evoked decrease in $[K^+]_o$. In response to brief light stimuli, which caused rod membrane responses having durations of <20 s, the solutions to this equation were very similar in waveform to the actual light-evoked decreases in $[K^+]_o$ (Matsuura et al., 1978; Oakley et al., 1979). In response to maintained illumination, which causes a maintained change in rod membrane voltage that can be approximated by a (negative) step (Oakley, 1983), the first-order MMT order predicts that $[K^+]_o$ should decrease exponentially to a minimum and remain at this value throughout the entire period of illumination. At light

offset, the MMT model predicts that $[K^+]_o$ should increase exponentially back to its dark value. Thus, the MMT model cannot account for the reaccumulation of K^+ during maintained illumination, nor can it account for the overshoot in $[K^+]_o$ following termination of maintained illumination.

A Revised Model

The activity of the Na^+/K^+ pump in most cells is known to depend on $[Na^+]_i$ (Skou, 1965; Thomas, 1972; Glitsch, 1972; Glynn and Karlish, 1975; Saito and Wright, 1982; Torre, 1982). Although Matsuura et al. (1978) recognized that the active transport of K^+ by the rods' Na^+/K^+ pump was likely to depend on both $[K^+]_o$ and $[Na^+]_i$, the model they actually tested assumed that the active transport of K^+ varied only as a function of $[K^+]_o$. Although this assumption simplified the model considerably, it was probably an oversimplification for two reasons. In many cell types, the Na^+/K^+ pump has binding sites for external K^+ that are half-saturated by $[K^+]_o$ in the region of 0.4–1.0 mM (Ostwald and Steinberg, 1980; Zeuthen and Wright, 1981; Gadsby, 1980; Saito and Wright, 1982), so that it seems unlikely that a small change in $[K^+]_o$ in the region of 1.0–3.0 mM (as observed in frog and toad) by itself would have a significant effect on pump activity. Moreover, rods have a large capability to transport Na^+ out of their interior (Hagins et al., 1970), so it is not unreasonable to think that large changes in $[Na^+]_i$ might occur in rods, with subsequent effects on the activity of their Na^+/K^+ pump. Thus, in revising the MMT model, it was logical to examine the effects of a light-evoked decrease in $[Na^+]_i$ upon the rods' Na^+/K^+ pump.[1]

The MMT model did not account for diffusion of K^+ through the extracellular space, but such diffusion is likely to be significant. The subretinal space normally is only several hundred microns away from the vitreous (a large reservoir of nearly constant $[K^+]_o$), and K^+ diffuses readily through the proximal retina (Steinberg et al., 1980). In the isolated retina preparation used in the present experiments, the subretinal space is adjacent to the bathing solution. It was found (Oakley, 1983) that the diffusion of K^+ from the bathing solution into the subretinal space was very rapid in the isolated retina preparation and could be characterized as a first-order process with a time constant of ~9 s. Consequently, diffusion of K^+ is likely to play an important role in determining the changes in $[K^+]_o$ in the subretinal space. It perhaps is worth noting that the spatial buffering of $[K^+]_o$ by glial cells is likely to be minimized in this preparation, since diffusion of K^+ through the extracellular space is so rapid.

In the Appendix, a revised version of the MMT model is derived in detail. It will be shown (below) that this revised model can account for the reaccumulation of K^+ during maintained illumination. This revised model accounts explicitly for the effects of $[Na^+]_i$ upon the rate of the rods' Na^+/K^+ pump and for

[1] It is possible that there also is a light-evoked increase in $[K^+]_i$ that might be expected to inhibit the pump (see Oakley, 1983, for details). Any terms involving $[K^+]_i$ were omitted from the model, since such a change in $[K^+]_i$ would probably occur on the same time scale as the change in $[Na^+]_i$, and recent evidence suggests that when both $[Na^+]_i$ and $[K^+]_i$ change, the Na^+/K^+ pump is much more sensitive to the change in $[Na^+]_i$ (Ballanyi et al., 1983).

diffusion of K^+ through the extracellular space. Specifically, the active transport of Na^+ and K^+ by the Na^+/K^+ pump is assumed to vary as a function of both $[Na^+]_i$ and $[K^+]_o$ (Skou, 1965; Glynn and Karlish, 1975; Saito and Wright, 1982). However, over the range of actual changes in $[K^+]_o$ known to occur in this preparation, the level of $[K^+]_o$ is assumed to be sufficiently high so that the activity of the pump is not altered significantly by the light-evoked changes in $[K^+]_o$ (Zeuthen and Wright, 1981; Gadsby, 1980; Saito and Wright, 1982). Thus, the active influx of K^+ is assumed to depend only on $[Na^+]_i$ (Eisner and Lederer, 1980; Torre, 1982).[2]

The revised model can be summarized briefly as follows. At the onset of maintained illumination, there is a step decrease in the passive influx of Na^+ entering the rod outer segments (caused by a decrease in membrane g_{Na}). In combination with this reduced influx of Na^+, the continuing activity of the rods' Na^+/K^+ pump produces a decrease in $[Na^+]_i$, which in turn produces a decrease in the rate of the pump (and thus a decrease in the active fluxes produced by the pump). At the onset of maintained illumination, there is also a step decrease in the passive efflux of K^+ out of the rod inner segment (caused by the membrane hyperpolarization). In combination with this reduced efflux of K^+, the continuing activity of the rods' Na^+/K^+ pump causes a decrease in $[K^+]_o$ in the subretinal space, which in turn causes K^+ to diffuse into the subretinal space from the bathing solution. As the rate of the pump slows (because of the decrease in $[Na^+]_i$), the diffusional flux of K^+ into the subretinal space exceeds the reduced removal of K^+ by the rods' Na^+/K^+ pump and this produces the reaccumulation of $[K^+]_o$. At light offset, the processes are reversed.

The revised model is represented by two equations (see Appendix) that describe the light-evoked changes in $[Na^+]_i$ and $[K^+]_o$. These equations are the solutions to a pair of coupled differential equations that express mathematically the assumptions of the model. The equation for $[Na^+]_i$ (Eq. 14) contains a single-exponential term having a rate constant k_1; since the pump rate is assumed to depend only upon $[Na^+]_i$, the decrease in pump rate during maintained illumination is also characterized by the rate constant k_1. The equation for $[K^+]_o$ (Eq. 15) contains two exponential terms, one having a rate constant k_1 (as in Eq. 14) and the other having a rate constant k_2, where k_2 is related to the time course of diffusion of K^+ through the extracellular space. In the following sections, the model is tested by comparing modeled responses with actual changes in $[K^+]_o$ under a wide range of stimulus conditions.

Testing the Revised Model

The rate constants k_1 and k_2 were varied in order to fit the model's response to actual measurements of $[K^+]_o$. Since V_{K^+} is a logarithmic measure of $[K^+]_o$, it was

[2] The first attempts at revising the MMT model included a term to account for the effects of changing $[K^+]_o$ on the pump rate. However, in order to fit the data, the coefficient of this term had to be very small. In fact, this term could be neglected with no significant effect on the modeled waveforms. Therefore, in the final version of the revised model, the effects of the light-evoked changes in $[K^+]_o$ on the pump rate have been neglected. It is possible that in an intact preparation, where $[K^+]_o$ changes over a larger range (e.g., Steinberg et al., 1980), the effects of $[K^+]_o$ may be greater.

necessary to convert the measurements of V_{K^+} to $[K^+]_o$. Using Eq. 2, it was possible to make this conversion for entire digitized waveforms of V_{K^+} (see Methods). In Fig. 8*A*, the linearized change in $[K^+]_o$ is shown that resulted from a 180-s period of maintained illumination (average of two responses). The modeled change in $[K^+]_o$ is shown in Fig. 8*B*. This waveform is given by Eq. 15, with $k_1 = 8.6 \times 10^{-3}$ s^{-1} and $k_2 = 0.155$ s^{-1}. These rate constants were chosen by trial and error to fit by eye the changes in $[K^+]_o$ during maintained illumination; that is, they were chosen to fit the initial time to peak and the time course of the reaccumulation. In Fig. 8*C*, the two waveforms have been superimposed (scaled for equal amplitudes of the light-evoked decrease in $[K^+]_o$). The modeled change in $[K^+]_o$ was extremely similar in waveform to the actual change in $[K^+]_o$. Moreover, although the rate constants were chosen to fit the change in $[K^+]_o$ during maintained illumination, the modeled response fits the overshoot of $[K^+]_o$ quite well, both in amplitude and in time course. The modeled change in $[Na^+]_i$ (Eq. 14, having $k_1 = 8.6 \times 10^{-3}$ s^{-1}) is shown in Fig. 8*D*. The model predicts that $[Na^+]_i$ should decrease over the entire 180-s period of maintained illumination. In order to fit the changes in $[K^+]_o$ observed in other experiments, k_1 varied from 6×10^{-3} to 9×10^{-3} s^{-1}, while k_2 varied from 0.15 to 0.18 s^{-1}.

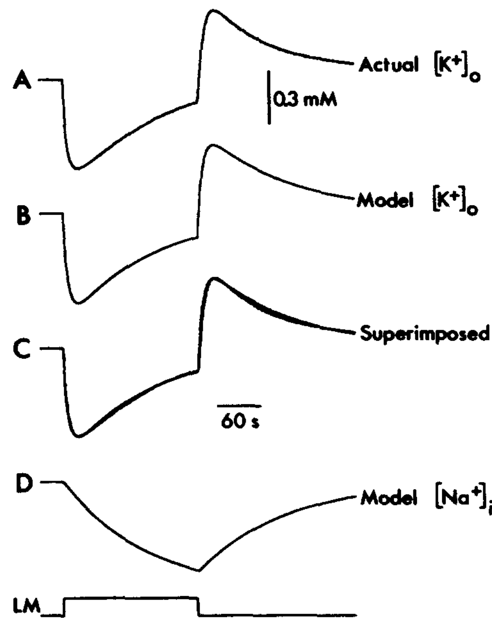

FIGURE 8. Testing the revised model. (*A*) Actual changes in $[K^+]_o$, linearized from measurements of V_{K^+}. This waveform is the average of two responses evoked by a 180-s light stimulus (indicated by the trace labeled LM). (*B*) Modeled changes in $[K^+]_o$, computed from Eq. 15 using $k_1 = 8.6 \times 10^{-3}$ s^{-1}, $k_2 = 0.155$ s^{-1}, and $t_1 = 180$ s. (*C*) The waveforms from parts *A* and *B* have been scaled for equal amplitudes of the light-evoked decrease in $[K^+]_o$ and superimposed. (*D*) Modeled change in $[Na^+]_i$, computed from Eq. 14, using the same parameters as in part *B*.

The fit between the model and the actual data was not always as good as in Fig. 8, since V_{K^+} sometimes contained random drift.

Additional Tests of the Revised Model

If the revised model is appropriate, then it should be able to predict the changes in $[K^+]_o$ evoked by brief stimuli, as well as the transition from the responses evoked by brief stimuli (having neither reaccumulation nor overshoot) to those responses evoked by maintained illumination (having both reaccumulation and overshoot). In Fig. 9, model responses to stimuli ranging from 15 to 240 s are shown, along with actual responses of $[K^+]_o$ (linearized from measurements of V_{K^+}). Again, the modeled responses (Fig. 9*B*) were very similar to the actual responses (Fig. 9*A*). For each type of response, the reaccumulation of K^+ and the overshoot developed as a similar function of stimulus duration. The changes in $[Na^+]_i$ predicted by the model also are illustrated (Fig. 9*C*).

If the revised model is a good one, then it should be able to predict changes in $[K^+]_o$ evoked by various types of stimuli that were not used in determining the coefficients of the model. In this respect, the fit to the overshoot in $[K^+]_o$ at light offset supports the revised model. In another such test of the revised model, 300-s periods of maintained illumination were interrupted by brief periods of

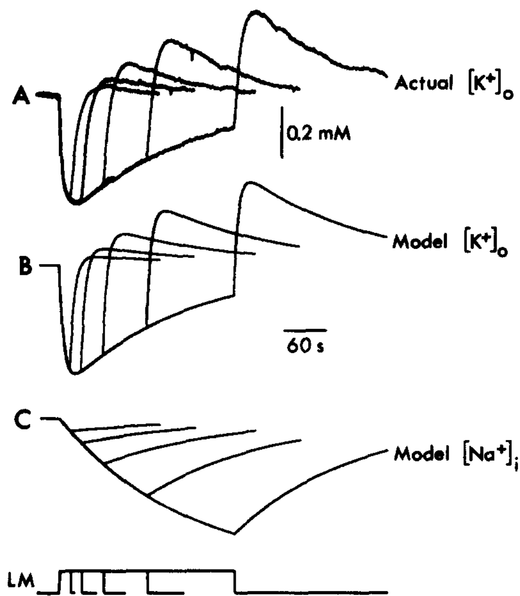

FIGURE 9. Effects of stimulus duration. (*A*) Actual changes in $[K^+]_o$, linearized from measurements of V_{K^+}. Superimposed are five waveforms, evoked by stimuli that were 15, 30, 60, 120, and 240 s in duration (indicated by the traces labeled LM). (*B*) Superimposed are five modeled changes in $[K^+]_o$. These waveforms were computed from Eq. 15 using $k_1 = 6.0 \times 10^{-3}\ s^{-1}$, $k_2 = 0.155\ s^{-1}$, and t_1 equal to 15, 30, 60, 120, and 240 s. (*C*) Modeled changes in $[Na^+]_i$. Superimposed are five waveforms that were computed from Eq. 14, using the same parameters as in part *B*.

darkness (5 or 20 s in duration), and the resulting changes in $[K^+]_o$ (linearized from V_{K^+}) are shown in Fig. 10*A*. In Fig. 10*B*, modeled responses are shown that were generated using similar periods of illumination and darkness. In support of the revised model, the fit between the modeled responses and the actual changes in $[K^+]_o$ was very reasonable. The modeled changes in $[Na^+]_i$ are shown in Fig. 10*C*.

The revised model can be tested further by seeing if it can account for the lack of reaccumulation and overshoot observed under certain experimental conditions. It was shown experimentally that the reaccumulation of K^+ during maintained illumination could be abolished by lowering the retinal temperature, superfusing with ouabain, lowering PO_2, or lowering $[K^+]_o$. All of these treatments should inhibit the rods' Na^+/K^+ pump and should also produce an increase in $[Na^+]_i$. In terms of the revised model, these effects will decrease the rate constant k_1 and also will decrease the maximum amplitude of the changes in $[K^+]_o$ (see Appendix).

To simulate inhibition of the pump, the active fluxes of Na^+ and K^+ in the dark were reduced (arbitrarily) to one-third of their control values. In addition,

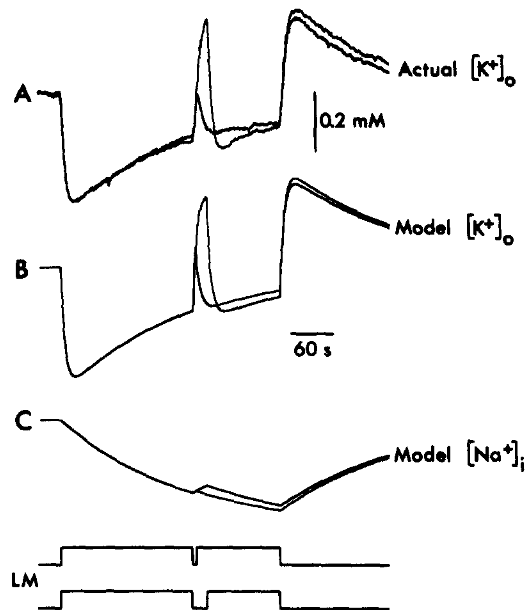

FIGURE 10. Effects of interrupted flashes. (*A*) Actual changes in $[K^+]_o$, linearized from measurements of V_{K^+}. Superimposed are two waveforms that were evoked by light stimuli that overall were 300 s in duration. These stimuli were interrupted by a 5- or 20-s period of darkness after the first 180 s (as indicated by the traces labeled LM). (*B*) Superimposed are two modeled changes in $[K^+]_o$. These waveforms were computed from Eq. 15, using $k_1 = 6.0 \times 10^{-3}$ s^{-1}, $k_2 = 0.175$ s^{-1}, and appropriate values of t_1 (using superposition, responses to several stimuli were summed algebraically). (*C*) Modeled changes in $[Na^+]_i$. Superimposed are two waveforms that were computed from Eq. 14, using the same parameters as in part *B*.

the sensitivity of the pump rate to changes in $[Na^+]_i$ was reduced to one-ninth of its control value (see Appendix, Fig. 12, point *B*). Together, these changes in the model's parameters had the effect of reducing k_1 from 8.60×10^{-3} (a typical value; see Fig. 8) to 9.56×10^{-4} s^{-1}. In Fig. 11*A*, control responses and responses simulating inhibition of the pump are superimposed. The model predicts that when the pump is inhibited, the light-evoked decrease in $[K^+]_o$, the reaccumulation of K^+ during maintained illumination, and the light-evoked decrease in $[Na^+]_i$ all will be reduced in amplitude. (It should be noted that if there is any reduction in the amplitude of the rod photoresponse when the pump is inhibited, this would cause an additional reduction in the amplitude of the light-evoked decrease in $[K^+]_o$.) The model also predicts that when the pump is inhibited, the level of $[K^+]_o$ will fall at a slower rate at light onset, because of the decrease in the rate of uptake of K^+ from the extracellular space. In Fig. 11*B*, the initial 70 s of the two simulated changes in $[K^+]_o$ have been scaled for equal peak amplitudes and superimposed. The model predicts that the magnitude of the initial slope of the normalized light-evoked decrease in $[K^+]_o$ will be only slightly less when the pump is inhibited. This result is due to the fact that the time course of the initial

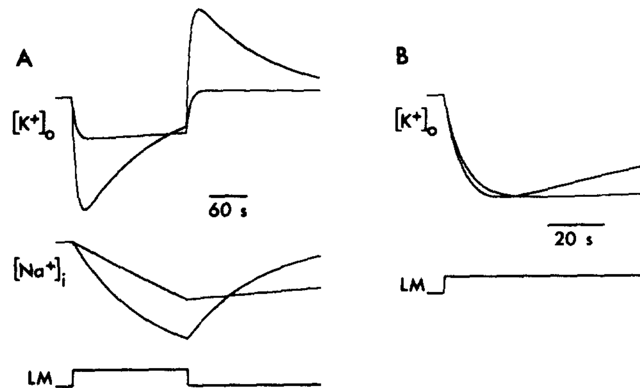

FIGURE 11. Simulated effects of inhibiting the Na^+/K^+ pump. (*A*) The modeled changes in $[K^+]_o$ and $[Na^+]_i$ that would result from a 180-s light stimulus (indicated by the trace labeled LM) were calculated using Eqs. 15 and 14, respectively. In order to observe more readily the effects of inhibiting the pump, the baselines of each type of response have been superimposed in the dark (even though the baseline level of $[K^+]_o$ changed when the pump was inhibited; see Figs. 3–6). For these responses, k_2 was 0.155 s^{-1} and t_l was 180 s. To simulate control conditions, k_1 was 8.6×10^{-3} s^{-1} (see Fig. 8). To simulate conditions that inhibit the pump, the coefficients a and J_{Na} both were reduced to one-third of their control values (see text), which decreased k_1 to 9.56×10^{-4} s^{-1} (one-ninth of its control value). Other values of these coefficients also were tested, with the result that the amplitude of the light-evoked decrease in $[K^+]_o$ was reduced in direct proportion to the reduction in J_{Na}, and negligible reaccumulation during a 180-s period of maintained illumination was observed when k_1 was reduced below 1.0×10^{-3} s^{-1}. (*B*) The first 70 s of each simulated change in $[K^+]_o$ have been scaled for equal peak amplitudes and superimposed.

part of the normalized response is dominated by diffusion for this particular set of rate constants k_1 and k_2. In support of the revised model, the simulated changes in $[K^+]_o$ in Fig. 11 are very similar to the actual data, as shown in Figs. 3–6.

Additional Features of the Revised Model

With brief stimuli that produce rod responses having durations of <20 s, the model predicts that the change in $[Na^+]_i$ will be small (see Fig. 9) and that there will not be a significant decrease in pump rate. Under these conditions, the rate constant k_2 will determine the waveform of the modeled decrease in $[K^+]_o$ (see Eq. 15, Appendix). The time constant, τ_2, of the initial portion of the modeled decrease in $[K^+]_o$ is $\tau_2 = 1/k_2 = 6.45$ s. This time constant is remarkably similar to the time constant of the light-evoked decrease in $[K^+]_o$ found earlier by Matsuura et al. (1978) in frog (9 s) and Oakley et al. (1979) in toad (5.5 s). However, Matsuura et al. (1978) interpreted this time constant as the time constant of the pump, whereas in the revised model, this time constant represents the diffusion of K^+ through the extracellular space (see Appendix). That this time constant represents diffusion is supported by the recent measurements of the time course of diffusion between the bathing solution and the subretinal space in the isolated retina of the toad. Using an approximate technique, it was found (Oakley, 1983) that such diffusion could be characterized as a first-order process having a time constant of ~9 s.

It is interesting to observe that in the MMT model, a decrease in $[K^+]_o$ leads to a decreased removal of K^+ from the extracellular space (because of inhibition of the pump), whereas in the revised model, a decrease in $[K^+]_o$ leads to an increased addition of K^+ into the subretinal space (because of an increase in the diffusional flux). Since both of these fluxes were assumed to vary linearly with the change in $[K^+]_o$, both of these fluxes produced analogous terms in the respective equations describing the decrease in $[K^+]_o$ in response to brief flashes, and it is not surprising that each term yielded a similar time constant.

The revised model assumes that diffusion supplies the flux of K^+ necessary for the reaccumulation of K^+ during the time periods when the active and passive fluxes of K^+ are reduced in magnitude. In the isolated retina preparation, the final level of $[K^+]_o$ reached during continuous illumination was essentially equal to the level of $[K^+]_o$ in the bathing solution (2.4 mM). In the intact preparations used earlier (cat, Steinberg et al., 1980; frog, Oakley and Steinberg, 1982), the final level of $[K^+]_o$ reached during maintained illumination was essentially equal to the level of $[K^+]_o$ at the vitreal border of the retina. These results are consistent with the revised model, since diffusion of K^+ can cause $[K^+]_o$ to reaccumulate only to the level in the surrounding extracellular spaces, and not above this level.

The revised model predicts that $[Na^+]_i$ should decrease significantly during maintained illumination. The size of the decrease in $[Na^+]_i$ cannot be predicted without knowing more about the dependence of the pump rate upon $[Na^+]_i$. However, the revised model predicts that the decrease in $[Na^+]_i$ will have a time constant $\tau_1 = 1/k_1 = 116$ s, so it should be a very slow process. Future experiments should use Na^+-selective microelectrodes to measure $[Na^+]_i$ (Cohen et al., 1982;

Ballanyi et al., 1983) in rods, in order to determine if there is indeed a slow decrease in $[Na^+]_i$ during maintained illumination.

The revised model also predicts that the rate of the Na^+/K^+ pump will decrease during maintained illumination. This prediction is supported by measurements of oxygen uptake. Kimble et al. (1980) found that frog rods decreased their consumption of oxygen during maintained illumination, as would be expected if the rate of their Na^+/K^+ pump was decreased.

Cat rods are much smaller than toad rods, and thus cat rods have a much larger surface-to-volume ratio than do toad rods. If both types of rods have a comparable density of Na^+/K^+ pump sites on their membranes, then cat rods should be able to decrease their $[Na^+]_i$ much more rapidly than toad rods, especially since the activity of their Na^+/K^+ pump is likely to be increased because of the higher temperature of the cat retina (37 vs. 22°C). Thus, the model predicts that the reaccumulation process should be much faster in cat than in toad. This prediction is supported by the experimental findings of Steinberg et al. (1980), who found that reaccumulation of K^+ in the subretinal space of the cat retina begins after only 4 s of maintained illumination and is essentially complete within 3 min (vs. 24 s and 6 min, respectively, in the toad retina at 22°C).

Finally, it must be mentioned that although the revised model supports a reasonable hypothesis regarding the mechanisms responsible for the observed changes in $[K^+]_o$ during and after maintained illumination, such support alone does not prove the hypothesis. Any model that can be represented by an equation of the form of Eq. 15 would produce similar behavior. The rate constants k_1 and k_2 were determined by curve fitting and as yet cannot be determined independently by experimentation. However, the revised model does provide a working hypothesis upon which to base the observations, and it is now clear that the predictions of the model, especially with regard to $[Na^+]_i$, must be tested experimentally.

APPENDIX

Derivation of the Revised Model

The model that is derived below is based upon the model originally proposed by Matsuura et al. (1978). In this revised model, primarily light-evoked changes in ionic fluxes will be considered, since these fluxes are the ones responsible for changing $[K^+]_o$. The active transport of Na^+ and K^+ by the Na^+/K^+ pump is assumed to vary as a function of both $[Na^+]_i$ and $[K^+]_o$ (Skou, 1965; Glynn and Karlish, 1975; Saito and Wright, 1982). However, over the range of actual changes in $[K^+]_o$ known to occur in this preparation, the level of $[K^+]_o$ is assumed to be sufficiently high so that the activity of the pump is not altered significantly by the light-evoked changes in $[K^+]_o$ (Zeuthen and Wright, 1981; Gadsby, 1980; Saito and Wright, 1982). Thus, the active influx of K^+ can be assumed to depend only on $[Na^+]_i$ (Eisner and Lederer, 1980; Torre, 1982). Active transport by the Na^+/K^+ pump is likely to vary as a function of $[Na^+]_i$ in a sigmoidal manner (e.g., Saito and Wright, 1982). However, for small changes about the dark level, the change in this active transport will vary linearly with the change in $[Na^+]_i$, termed Na, as shown in Fig. 12 (upper curve, point *A*). Therefore, the active efflux of Na^+ will vary according to the relationship:

$$\text{active efflux} = (1 + a\text{Na})\, J_{\text{Na}}, \tag{3}$$

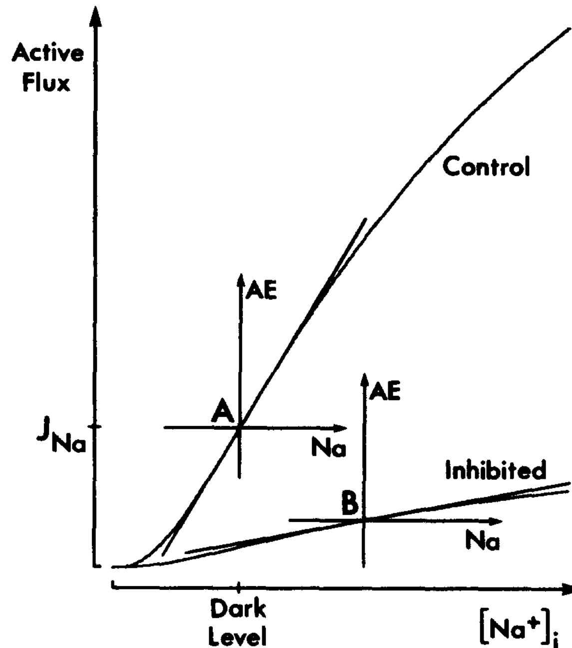

FIGURE 12. The assumed relationship between the active flux of Na^+ (or K^+) and $[Na^+]_i$. The solid curves are of the form:

$$J = J_{max}\left(1 + \frac{K_m}{[Na^+]_i}\right)^{-3},$$

where J is the active flux, J_{max} is the maximum value of J, and K_m is the affinity constant of the internal binding site for Na^+ (Eq. 6; Saito and Wright, 1982). Under control conditions (upper curve, labeled "Control"), the system operates in the dark at point A, with an active efflux of Na^+ equal to J_{Na} and a level of $[Na^+]_i$ that is approximately equal to K_m (Saito and Wright, 1982). In this example, the dark level of $[Na^+]_i$ was taken to be 7 mM (Torre, 1982). With respect to these values in the dark, the change in the active efflux of Na^+ is termed AE and the change in $[Na^+]_i$ is termed Na (the positive directions for AE and Na are indicated on the axes drawn through point A). For small variations about point A, AE is linearly related to Na, as indicated by the straight line drawn through point A. The slope of this line is equal to the quantity aJ_{Na} (see Eq. 4), where the coefficient a is the slope of the line when J_{Na} is normalized to unity (thus, J_{Na} scales the coefficient a to account for the actual flux). When the Na^+/K^+ pump is partially inhibited, the active flux should decrease for any given $[Na^+]_i$ (lower curve, labeled "Inhibited"), the level of $[Na^+]_i$ should increase, and the system (hypothetically) would operate at point B. At point B, J_{Na} was reduced to one-third of its control value and the slope of the straight line relating AE and Na was reduced to one-ninth of its control value (see text).

where J_{Na} is the magnitude of the flux of Na^+ in the dark and the coefficient a is a measure of the sensitivity of the active flux to changes in $[Na^+]_i$. The light-evoked change in the active efflux of Na^+ out of the rod, termed AE, thus can be written as:

$$AE = aJ_{Na}\ \text{Na} \qquad (4)$$

(see the legend to Fig. 12). It is implicit in this model that the rod is in a steady state in the dark; that is, the active efflux of Na^+ in the dark is equal in magnitude to the passive influx of Na^+ in the dark (and both are equal in magnitude to J_{Na}).

At light onset ($t = 0$), the light-evoked decrease in g_{Na} will cause a decrease in the passive influx of Na^+ that is continuously entering the rod down its electrochemical gradient (Hagins et al., 1970). In the simplest case, the passive influx will be large in the dark and will be reduced in the light. With maintained illumination, the change in rod membrane voltage, V, will be approximated as a negative step ($V = 0$ for $t < 0$; $V = -1$ for $t \geq 0$). Thus, the magnitude of the change in the passive influx of Na^+ into the rod, termed PI, can be written as:

$$PI = bVJ_{Na}, \tag{5}$$

where b is a constant ($0 < b < 1$).

The rate of change of $[Na^+]_i$ is given by the change in the net flux of Na^+ into the cell, divided by the intracellular volume, vol_i, which can be written as:

$$\begin{aligned} \frac{d\text{Na}}{dt} &= \frac{1}{vol_i}(PI - AE) \\ &= \frac{k_1 bV}{a} - k_1\text{Na}, \end{aligned} \tag{6}$$

where $k_1 = \frac{aJ_{Na}}{vol_i}$. The solution to this first-order differential equation is:

$$\text{Na} = V\frac{b}{a}(1 - e^{-k_1 t}). \tag{7}$$

This equation states that $[Na^+]_i$ should fall exponentially after the onset of maintained illumination (that is, in response to a negative step, V). Moreover, the active transport of Na^+ (and thus of K^+) should decrease with this same time dependence.

For K^+, the differential equation describing $[K^+]_o$ is coupled to the equation describing $[Na^+]_i$, since the active transport of K^+ is assumed to depend only on $[Na^+]_i$. Assuming that the pump is electrogenic (Torre, 1982), then the magnitude of the active fluxes of K^+ and Na^+, as well as the changes in these active fluxes, will be related by a constant (multiplicative) factor. For example, it seems that the ratio of the active efflux of Na^+ to the active influx of K^+ is ~6:5 in rods (Torre, 1982). Thus, the light-evoked change in the active influx of K^+, termed AI, can be written as:

$$AI = a\text{Na}J_K = bV(1 - e^{-k_1 t})J_K, \tag{8}$$

where J_K is the flux of K^+ in the dark. The ratio of J_{Na} to J_K will depend upon the electrogenicity of the pump (e.g., 6:5).

The light-evoked hyperpolarization of the rod will decrease the driving force on K^+, which will in turn decrease the passive efflux of K^+ from the rod. In this simplified model, the change in the passive efflux of K^+ out of the rod, termed PE, will vary according to:

$$PE = cVJ_K, \tag{9}$$

where c is a constant ($0 < c < 1$). As with Na^+, it is assumed that the rod is in a steady state with respect to K^+ in the dark; that is, the active influx of K^+ in the dark is equal in magnitude to the passive efflux of K^+ in the dark (and both fluxes are equal in magnitude to J_K).

In the isolated retina, K^+ will diffuse into the subretinal space from the bathing solution (in the intact eye, K^+ will diffuse from the proximal retina and the vitreous, and it also may diffuse from the choroid into the subretinal space across the paracellular junctions of the retinal pigment epithelium). The magnitude of the flux of K^+ caused by diffusion will be proportional to the concentration gradient between the bathing solution and the subretinal space. Since the bathing solution has a constant $[K^+]$, the concentration gradient will vary linearly with the change in $[K^+]_o$ in the subretinal space, termed K, and the light-evoked change in the diffusional flux, termed J_D, can be approximated as:

$$J_D = -d\mathrm{K}, \tag{10}$$

where d is a constant.

The rate of change of $[K^+]_o$ in the subretinal space will be given by the change in the net flux of K^+ into this space, divided by the effective extracellular volume, vol_o, and can be written as:

$$\begin{aligned} \frac{d\mathrm{K}}{dt} &= \frac{1}{vol_o}(J_D + PE - AI) \\ &= -k_2\mathrm{K} + fV + gVe^{-k_1t}, \end{aligned} \tag{11}$$

where $k_2 = \dfrac{d}{vol_o}$, $f = \dfrac{(c-b)J_K}{vol_o}$, and $g = \dfrac{bJ_K}{vol_o}$.

The solution to this equation is:

$$\mathrm{K} = \frac{gV}{k_2 - k_1}(e^{-k_1t} - e^{-k_2t}) + \frac{fV}{k_2}(1 - e^{-k_2t}). \tag{12}$$

Since the change in $[K^+]_o$ in this preparation is very small when stimuli of very long duration are used (Oakley, 1983), this means that the term fV/k_2 must be very small, which implies that the coefficient f is nearly zero. Therefore, the term involving f can be omitted with no significant effect on the model, and the equation describing the change in $[K^+]_o$ finally can be written as:

$$\mathrm{K} = \frac{gV}{k_2 - k_1}(e^{-k_1t} - e^{-k_2t}). \tag{13}$$

The above equation describes the step response of an overdamped, second-order system. In order to have a light-evoked decrease in $[K^+]_o$, k_2 must be larger than k_1. Thus, k_2 determines the initial rate of change of the light-evoked decrease in $[K^+]_o$, while k_1 determines the rate of reaccumulation of K^+ during maintained illumination. Each of these rate constants contains a term involving either the intracellular volume or the extracellular volume and thus changes in volume fraction (for example, because of cell swelling) during maintained illumination would affect these constants. However, in preliminary experiments using the impermeant cation TMA^+ and TMA^+-selective microelectrodes (experiments similar to those described by Dietzel et al., 1980, and Connors et al., 1982), no changes in extracellular volume (and therefore volume fraction) were observed (H. Shimazaki and B. Oakley, unpublished observations). Thus, it is not likely that the value of either rate constant changes significantly during maintained illumination.

The above analytical solutions for the changes in $[Na^+]_i$ and $[K^+]_o$ (Eqs. 7 and 13) are valid only for the time period following the onset of maintained illumination. These solutions are not valid for the time period following the offset of maintained illumination. At the offset of a maintained stimulus having duration t_1 s, the model's responses will

be given by the superposition of two responses, one to a negative step, V, at light onset ($t = 0$), and the other to a positive step starting at light offset ($t = t_1$). The positive step, V', will be equal to 0 for $t < t_1$ and will be equal to +1 for $t \geq t_1$. Both of these steps are assumed to have infinite duration. The complete solution for the changes in $[Na^+]_i$ and $[K^+]_o$, both during and after maintained illumination, thus can be written as:

$$\mathrm{Na} = V\frac{b}{a}(1 - e^{-k_1 t}) + V'\frac{b}{a}[1 - e^{-k_1(t-t_1)}]; \tag{14}$$

$$\mathrm{K} = \frac{g}{k_2 - k_1}\{V[e^{-k_1 t} - e^{-k_2 t}] + V'[e^{-k_1(t-t_1)} - e^{-k_2(t-t_1)}]\}. \tag{15}$$

Eqs. 14 and 15 can be used to compute the waveforms of the changes in $[Na^+]_i$ and $[K^+]_o$ (see Discussion). The revised model provides an exact (analytical) solution to the changes in $[K^+]_o$ and $[Na^+]_i$. There is no need to integrate the differential equation numerically (as done previously by Oakley et al., 1979) or to simulate the differential equation using an analog circuit (as done previously by Matsuura et al., 1978).

Effects of Inhibiting the Na^+/K^+ Pump in the Rods

Inhibiting the Na^+/K^+ pump in rods should produce an increase in $[Na^+]_i$. If this is the case, then inhibiting the pump will decrease the active fluxes of K^+ and Na^+ in the dark (J_K and J_{Na}), as well as decrease the linearized slope (aJ_{Na}) of the sigmoidal curve relating the change in the active flux of Na^+ (or K^+) to the change in $[Na^+]_i$ (Fig. 12, lower curve, point B). In turn, the changes in these parameters will decrease both k_1 and the coefficient g, and thus will produce a smaller change in $[K^+]_o$, having less reaccumulation. In the limit as k_1 approaches zero, the equation describing the change in $[K^+]_o$ will be uncoupled from the effects of $[Na^+]_i$, and there will be no reaccumulation of K^+ during maintained illumination.

To simulate inhibition of the pump, the fluxes J_{Na} and J_K were reduced (arbitrarily) to one-third of their control values (the active transport of Na^+ and K^+ in the dark should be reduced if the pump is inhibited), and the linearized slope (aJ_{Na}) of the sigmoidal curve was reduced to one-ninth of its control value (Fig. 12, lower curve, point B). Together, these changes in the model's parameters had the effect of reducing k_1 from 8.60×10^{-3} (a typical value; see Fig. 8) to $9.56 \times 10^{-4}\ s^{-1}$ and reducing the coefficient g (a scale factor) to one-third of its control value. These values are used in testing the model in Fig. 11.

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